



Influence of PFDA on the nutrient removal from wastewater by hydrogels containing microalgae-bacteria

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ABSTRACT

PFAS have demonstrated to affect some aerobic microorganisms applied for wastewater treatment. This study evaluated the nutrient removal of three types of hydrogels containing a consortium of microalgae-bacteria (HB), activated carbon (HC), or both (HBC) in presence of perfluorodecanoic acid (PFDA). The nutrients evaluated were ammonium nitrogen ($\text{NH}_4\text{-N}$), nitrate nitrogen ($\text{NO}_3\text{-N}$), phosphate (PO_4), and chemical oxygen demand (COD). Fluorine (F^-) concentration and the integrity of HB exposed to PFDA were also determined at the end of experiments to understand the potential sorption and effects of PFDA on hydrogel. The results indicated that the presence of PFDA did affect the nitrification process, 13% and 36% to HB and HBC, respectively. Mass balance confirmed negative impact of PFDA on nitrogen consumption in HB (-31.37%). However, $\text{NH}_4\text{-N}$ was removed by all types of hydrogels in a range of 61–79%, while PO_4 was mainly removed by hydrogels containing activated carbon (AC), 37.5% and 29.2% for HC and HBC, respectively. The removal of both NH_4 and PO_4 , was mainly attributed to sorption processes in hydrogels, which was enhanced by the presence of AC. PFDA was also adsorbed in hydrogels, decreasing its concentration between 18% and 28% from wastewater, and up to 39% using HC. Regarding COD concentration, this increased overtime but was not related to hydrogel structure, since Transmission Electron Microscopy imaging revealed that their structure was preserved in presence of PFDA. COD increase could be attributed to soluble algal products as well as to PVA leaching from hydrogels. In general, the presence of AC in hydrogels can contribute to mitigate the toxic effect of PFDA over microorganisms involved in biological nutrient removal, and hydrogels can be a technique to partially remove this contaminant from aqueous matrices.

1. Introduction

The current water quality deterioration increases day by day because of the increasing number of pollution sources. Moreover, high loads of conventional pollutants, e.g., carbon, nitrogen and phosphorous, and emerging pollutants limit the efficiency of conventional

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wastewater treatment plants (WWTPs) [1–3]. These last pollutants do include a wide range of contaminants of emerging concern (CECs), which can be present at different levels depending on the source of the wastewater [4,5]. CECs are unregulated water chemicals, which may cause ecological and human health impacts [6]. CECs are incorporated in personal care products, pesticides, pharmaceuticals, and industrial chemicals, among others. For example, per- and polyfluoroalkyl substances (PFAS) are synthetic organofluorine compounds that have an anionic functional group, as well as a perfluoroalkyl chain, which makes them a water and oil repellent [7]. Because of these properties, the use of PFAS at domestic and industrial level has increased dramatically in recent decades. Common uses for PFAS include applications as flame retardant and anti-adherent in household products, such as carpets, paper, and non-stick cookware, and even coated cardboard takeout containers (Table S1, Supplementary Information) [8]. As a part of the life cycle of these products, PFAS are released to wastewater, reaching other environmental matrices such as groundwater and surface water resources [9,10].

The structure, chain length and functional groups of each type of PFAS have been related to their distribution in the environment [11,12]. For example, PFAS with 8 or less carbon (short chain) can be mainly found in surface waters, while chains with more than 8 carbon (long chain) can travel via water streams and bioaccumulate in fish tissues and sediments [7,13,14]. As it can be seen in Table S1, as PFAS chain increases, melting and boiling points also increase, and then, the potential degradation of PFAS can also require a greater amount of energy. In fact, the energy required to break down the C–F bond is < 544 kJ/mol [15], which is high and provides to PFAS high resistance to chemical and biological degradation during any technology applied for water or wastewater treatment [16,17].

Lewis et al. [8] classified water treatment technologies for PFAS into destructive and adsorptive methods. The last techniques, despite not being able to degrade PFAS, can effectively remove these specific compounds from the aqueous media via physisorption or chemisorption [18]. These techniques include the use of polymers and activated carbon (AC), as well as the addition of specific proteins [8]. The destructive techniques include photocatalysis [19] such as photocatalytic ozonation [20] and photocatalytic decomposition [21], sonolysis [22], hydrothermal alkaline treatment [23], electrochemical oxidation [24], among other techniques that result in the reduction of the chain length, and the break of C–F bond. However, no attention has been given to the release of fluorine (F⁻) from destructive processes [25], which can compromise microorganisms in aquatic and environmental matrices. Actually, F⁻ induces toxicity to bacteria at levels of 0.1–1.9 mg/L by inhibiting and interfering metabolic activities [26]. Not only F⁻ represents a risk, PFAS themselves are xenobiotic substances that limit bacterial performance under aerobic conditions [27], and have presented microbial selectivity in soil microorganisms during chronic exposure (60 days) at µg-level [28].

Also in aerobic conditions, perfluorooctane sulfonate (PFOS), a type of PFAS, has altered the structure, functionality, and key enzyme activities of activated sludge microbial community at chronic exposure of concentrations <1 mg/L, during of 48 days [29]. Other effects of PFAS during aerobic wastewater treatment did include an 8.3% decrease in chemical oxygen demand (COD) removal [30]. For anaerobic conditions [31], reported the inhibition of up to 19% in methane production, and the decreasing of acidogens and methanogens at 30–60 µg/g TS during the anaerobic digestion of waste sludge. These studies suggest the adverse effects of PFAS over microorganisms involved in conventional WWTPs. However, reports about their effects in microorganisms embedded in new technologies such as carriers, beads, hydrogels among others applied for wastewater treatment are scarce. These new treatment technologies have been studied and applied to complement or to replace conventional WWTPs, aiming the reduction of hydraulic retention time [32], and total process energy duties [33], as well as increasing the removal of CECs.

The immobilization of microorganisms in polymeric matrices, such as hydrogels, is a biotechnological strategy that, compared to suspended biomass systems, addresses technical and economic limitations [34], such as high energy consumption for aeration required for nitrification [35], management of excess biomass [36], and poorly separation of liquid and solid in the sedimentation tank [37]. In contrast, immobilization of microorganisms in hydrogels allows to separate biomass easily [38], provide protection to microorganisms against shear stress and toxic compounds in aqueous media [39], and reduce the cost of aeration while facilitate the mass transfer involved in the removal of conventional contaminants [40].

Determination of the effect of CECs, such as PFAS, on microorganisms embedded in hydrogels during nutrient removal in wastewater treatment is important to understand the tolerance of this technology to such contaminants. Thus, the aim of this study was to evaluate the impact of PFDA - a PFAS frequently used in industry with applications as wetting agent and flame retardant - on the performance of hydrogels containing either a microalgae-bacteria consortium, AC, or both during nutrient removal processes from wastewater.

2. Materials and methods

2.1. Experimental setup

The experiment consisted of the exposure of three types of hydrogels to four media while the evaluation of main nutrients removal was performed. The types of hydrogels varied regarding the materials embedded, which were a consortium of microalgae-bacteria (HB), AC (HC < 45 µm, HYCEL), and both (HBC). All types of hydrogels were evaluated for nutrient and PFDA removal in four different media, such as synthetic wastewater (SWW), SWW with PFDA, PFDA solution, and DI water as control. Nutrients evaluated were ammonium nitrogen (NH₄-N), nitrate nitrogen (NO₃-N), phosphate (PO₄), and COD. PFDA concentrations were also evaluated as response variable for each treatment. Experiments were performed in 125 mL Erlenmeyer flasks during 72 h. Each flask contained 11 g of hydrogels and 89 g of each medium. Flasks were kept in an incubator (PSI AlgaeTron AG130) at an average temperature of 27 ± 1 °C, under constant stirring (150 rpm). Experiments were irradiated with white LED light at 160 µmol/m²/s, with a cycle of 12 h of light and 12 h of darkness. The pH, electrical conductivity (EC), dissolved oxygen (DO) and temperature were monitored every 24 h in

each treatment. Measurements of parameters such as $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, PO_4 , and COD, were performed per triplicate at initial time and every 24 h, for a total period of 72 h; while PFDA content was evaluated at time 0 and 72 h. To study the influence of PFDA in $\text{NH}_4\text{-N}$, ammonium removal efficiency (ARE) (Eq. (1)) was determined in experiments containing SWW and PFDA [41]:

$$\text{ARE} = \left[1 - \left(\frac{\text{NH}_4 \text{ Final}}{\text{NH}_4 \text{ Initial}} \right) \right] \times 100 \quad (1)$$

The nitrification rate per treatment was calculated based on the variation of nitrate concentration every 24 h in a lapse of 72 h. Equations (2)–(4) were used to determine each nitrification rate for all treatments. This was done by considering the equivalent fraction of the atomic weight of nitrogen in the nitrate molecule (0.226), where N_1 = nitrate concentration (mg/L), C_1 = nitrate concentration produced (mg/L), N_2 = net nitrogen removal (mg N), v = experimental volume (L), t represents time (h) and TSS (g) the mass of microorganisms present. Nitrogen mass balance was carried out according to Eq. (5).

$$N_1 = 0.226 \cdot C_1 \quad (2)$$

$$N_2 = N_1 \cdot v \quad (\text{Eq. } 3)$$

$$\text{Nitrification rate} \left(\frac{\text{mg N}}{\text{g TSS} \cdot \text{h}^{-1}} \right) = \frac{N_2}{\text{TSS} \cdot t} \quad (4)$$

$$\text{Nitrogen Consumption (\%)} = \left[\frac{(\text{NH}_4 - N_{\text{Initial}} - \text{NH}_4 - N_{\text{Final}}) - (\text{NO}_3 - N_{\text{Final}})}{\text{NH}_4 - N_{\text{Initial}}} \right] \cdot 100 \quad (5)$$

2.2. Hydrogel preparation

The three types of hydrogels namely, HB, HC, and HBC, were synthesized in our experiment. All hydrogels were synthesized following the procedure previously reported [42], and just adding inoculum (consortium microalgae-bacteria), AC (size < 45 μm , HYCEL), or both. Briefly, hydrogels were produced from a solution polyvinyl alcohol (PVA, $(\text{C}_4\text{H}_6\text{O}_2)_n$, Kuraray), sodium alginate (SA, $(\text{C}_6\text{H}_7\text{NaO}_6)_n$, Sigma Aldrich) and a fraction of biomass in deionized water (DI). The solution was autoclaved for 15 min at 121 $^\circ\text{C}$. Subsequently, the solution was dripped into a crosslinking solution, of boric acid (H_3BO_3 , Sigma Aldrich) and calcium chloride (CaCl_2 , Hycl), The resulting beads were kept in the crosslinking solution for 24 h, under constant stirring at 150 rpm. Finally, the hydrogel beads were transferred to a sodium sulfate (Na_2SO_4 , Qmed Natural) buffer and stirred for 24 h (150 rpm).

Inoculum of the consortium of microalgae-bacteria was collected from adapted culture of enriched microalgae-nitrifying bacteria, which consisted of *Chlorella* sp., *Scenedesmus* sp., and diatoms, which were used owing to their ability to grow in wastewater and nitrifying sludge from WWTP. This ability was promoted during a period of 60 days prior to the experiment used as adaptation process of culture. During this period, 4 Erlenmeyer flasks of 1 L operated as sequencing batch reactors (SBR). Each flask contained 400 mL of seed culture and wastewater (400 mL), which was replaced every three days. Wastewater and nitrifying bacteria were collected from the WWTP at Tecnológico de Monterrey Campus Puebla, which is based on extended aeration. Flasks were placed in an incubator (PSI AlgaeTron AG130) at an average temperature of 27 $^\circ\text{C}$ under constant stirring at 150 rpm and receiving white LED light radiation at an intensity of 160 $\mu\text{mol}/\text{m}^2 \cdot \text{s}$, with 12 h light and 12 h darkness cycle. Adapted consortium was characterized every 3 days as a function of pH, DO, EC, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and COD. For incorporation of consortium to hydrogels, an aliquot of the adapted culture was centrifuged at 1792 G, at 27 $^\circ\text{C}$ for 20 min 5 g of wet biomass was incorporated to a solution containing 10 wt% of PVA and 2 wt% of SA. To obtain hydrogels with AC, 0.44 g of AC was added to hydrogel solution. The resulting solution of each type of hydrogel was dripped into a crosslinking solution, containing 5.6 wt% of boric acid and 2 wt% of calcium chloride, under constant stirring at 150 rpm. After 24 h of synthesis, hydrogels were transferred to a 7.1 wt% sodium sulfate buffer (pH 6.29), keeping under constant stirring (150 rpm) for 48 h. Finally, each type of hydrogel was distributed and put into each of the 4 media for experimentation.

2.3. Media composition

Three different media were prepared to test the hydrogels removal processes. Control consisted of DI water, and it was used to analyze the behavior of hydrogels over time without any interference. The first medium was SWW (medium A) and was used to emulate the composition of wastewater, since real WW varies daily, with SWW is possible to minimize its variation and have greater control of conditions [43]. SWW contained (mass per liter): 951.10 mg glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, Caisson), 321.25 mg ammonium chloride (NH_4Cl , Amresco), 49.13 mg dipotassium hydrogen phosphate (K_2HPO_4 , Hycl), 44.21 mg magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Jalmek), 18.56 mg calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Hycl), 71.40 mg sodium chloride (NaCl , Meyer), 0.18 mg magnesium chloride tetrahydrate ($\text{MgCl}_2 \cdot 4\text{H}_2\text{O}$, Amresco), 0.29 mg H_3BO_3 , 0.10 mg zinc chloride (ZnCl_2 , Meyer), 0.27 mg iron (III) chloride (FeCl_3 , Meyer), and 0.28 mg edetic acid ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$, Amresco). For the preparation of the SWW with PFDA (media B) and third medium just with PFDA (media C), a solution of 100 mg/L of PFDA ($\text{C}_{10}\text{HF}_{19}\text{O}_2$, Sigma Aldrich) in DI water was used. A final concentration of 50 mg/L of PFDA was used to exemplify an acute exposure scenario of hydrogels to the pollutant. First, the PFDA was dissolved in 600 mL of DI water in a hot-plate magnetic stirrer, under constant stirring (200 rpm) at 80 $^\circ\text{C}$ until it was completely dissolved. Subsequently, the stock solution was divided into two flasks to obtain a concentration of 50 mg/L in both solutions. One solution was mixed with 300 mL of SWW to obtain medium B. The remaining 300 mL were diluted with 300 mL of DI water for the medium C (Table 1). Media A and

B were used to analyze the influence of PFDA during the nutrient removal processes. Main characteristics of all media are shown in Table 1, where the variations in characteristics between medium A and B could result from the different functional groups of PFDA interacting with ions present in the SWW, which modifies the availability of certain nutrients.

2.4. Analytical methods

The measurements of pH, EC and temperature were done with a portable meter using the corresponding probes (HQd, HACH). DO values were monitored with Consort 6010 Multiparameter Portable Analyzer. In all samples, the content of COD, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and PO_4 were analyzed according to the commercial methods (HACH). A solar radiation meter (PCE-SPM 1) was used to measure light intensity directly. The determination of TSS, VSS, TS, TVS in the consortium was done according to Standard Methods (2540, [44]). PFDA concentration in samples was indirectly determined as fluoride (F^-), after sulfuric acid distillations following the procedure of the standard methods (4500-B, [44]). In this late case, sulfuric acid was distilled before experiments to remove fluoride contamination, and to adjust the acid-water ratio for subsequent distillations in samples as suggested by the method. F^- concentration was quantified in samples using the SPADNS reagent (HACH). Briefly, in a distilling flask with glass beads, 13.3 mL of DI water was added. Then, 8 mL of sulfuric acid was carefully added. The solution was heated until it reached 180 °C (because of heat retention by the mantle and to prevent overheating, heating was stopped when temperature reached 178 °C). Distillate was recovered and discarded. Once the acid mixture had cooled to 80 °C or below, 10 mL of sample was added. With stirrer operating, the solution was heated once again until it reached 180 °C (heat was turned off before 178 °C to prevent sulfate carryover). The distillate of each sample was transferred to 50 mL Falcon tubes at room temperature, where each sample was gauged with 40 mL of DI water. Finally, each sample was poured into a 50 mL beaker to apply a SPADNS reagent (HACH). After mixing the content of the ampul containing SPADNS with the sample for 1 min, they were read to obtain the concentration of fluoride (F^-). To get the final concentration of PFDA per sample, the results were multiplied by dilution factor. Subsequently, the data were compared with the initial concentration based on the equivalent molecular weight of F^- in the PFDA molecule. This method was validated by measuring the recovery rate of F from DI water and SWW, which were 88.6% and 93%, respectively. Sample preparation for TEM imaging was performed according to Ref. [43].

2.5. Statistical analysis

Aqueous media (SWW and PFDA solution) and type of hydrogel (HB, HC and HBC) were the main factors analyzed with their respective levels. The effect of the levels of each factor on the response variables was analyzed through principal effects plot. Therefore, a 3×3 factorial design was carried out with Minitab using a p-value of 0.05, where the response variables were nutrient concentrations such as COD, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and PO_4 , as well as PFDA. Results of response variables are shown as the average value \pm standard deviation.

3. Results

3.1. Nitrogen removal

3.1.1. Ammonium removal

The variation of $\text{NH}_4\text{-N}$ concentration over time in media containing SWW and three types of hydrogels is shown in Fig. 1. In hydrogels exposed only to SWW, which had an initial concentration of 83 mg/L of $\text{NH}_4\text{-N}$, HB achieved the highest removal of ammonium (73%) at the end of the experiment, followed by HBC with 70.4% and HC with 64.2%. For SWW with PFDA, the best results for ammonium removal were found in hydrogels with AC, HBC with 76.8% and HC with 74.3% (Fig. 1). Hydrogels with only biomass (HB) presented the lowest ammonium removal (66.3%) at 72 h (Fig. 1). Statistical analysis evidenced that the $\text{NH}_4\text{-N}$ concentration varied significantly with the type of hydrogel used, presenting higher removal in presence of AC. Hence, $\text{NH}_4\text{-N}$ could be mainly removed via adsorption. Regarding the presence of PFDA, it was observed that there were no notable differences, indicating that the PFDA did not influence the removal of $\text{NH}_4\text{-N}$ (Fig. S1).

Table 1

Physicochemical parameters of all media used in the experiments: DI water, synthetic wastewater (SWW), SWW with perfluorodecanoic acid (PFDA), and PFDA solution. Note: BDL means below detection limit.

Parameter	DI water (control)	SWW (A)	SWW with PFDA (B)	PFDA solution (C)
pH	5.8	7.19	6.05	4.80
EC ($\mu\text{S}/\text{cm}$)	≤ 1	1080	546	35.8
DO (mg/L)	0.9	6.6	7.7	7.3
Temperature (°C)	21.7	21.8	21.6	21.6
COD (mg/L)	BDL	804 ± 1.04	386 ± 1.04	4 ± 1.04
$\text{NH}_4\text{-N}$ (mg/L)	0.18	83 ± 5.8	36.64 ± 3.2	0.58 ± 0.2
$\text{NO}_3\text{-N}$ (mg/L)	BDL	BDL	BDL	BDL
PO_4 (mg/L)	BDL	8.81 ± 1.8	4.5 ± 0.8	BDL
F^- (mg/L)	–	–	36.1	36.1

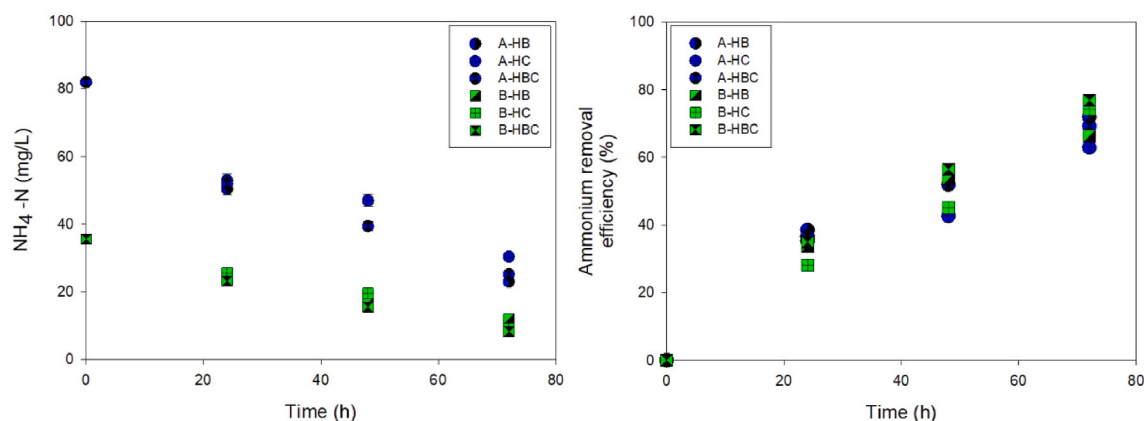


Fig. 1. Time course change in NH₄-N concentration (Left) and Ammonium Removal Efficiency (ARE) (Right) for different hydrogels: HB: hydrogel with biomass, HC: hydrogel with AC, HBC: hydrogel with biomass and AC, exposed to medium A (synthetic wastewater) and medium B (synthetic wastewater with PFDA).

3.1.2. Nitrate production

The NO₃-N production was different for all hydrogels in SWW media over time (Fig. 2). The initial concentration for all treatment was below the detectable level by the method used (<0.1 mg/L). For experiments with only SWW, the highest nitrate productions were from hydrogels with biomass, HBC with 43 mg/L and HB with 40 mg/L, which is expected due to the ability of the consortium to perform the nitrification process [41]. In the case of hydrogels exposed to SWW with PFDA, HB achieved the highest production at the end of the experiment with 34.8 mg/L, followed by HBC with 27.4 mg/L. The results coincided with the nitrification rate of hydrogels containing the consortium and exposed to both media (Fig. 2). Hydrogels with biomass (HB & HBC) and exposed to SWW presented a mean nitrification rate of 0.85 mg N/g TSS h, while that for same hydrogels exposed SWW with PFDA was 0.37 mg N/g TSS h, which proves that the presence of PFDA inhibited the consortium for nitrate production. Statistical analysis indicated that biomass enhanced the nitrification in hydrogels and confirmed that PFDA did have a negative effect on nitrate production (Fig. S2).

3.2. Phosphate removal

Variation of phosphate concentration over time for all hydrogels is shown in Fig. 3. For SWW, the initial concentration was 8.2 mg/L and HB achieved the highest phosphate removal (95.1%), while HBC resulted in the lowest removal (7.3%). For SWW with PFDA, hydrogels with AC obtained the highest removal, 37.5 and 29.2%, for HC and HBC, respectively, while HB removed only 4.2%. Statistical analysis indicated that AC enhanced the PO₄ removal, while the principal effects plot confirmed that PFDA negatively affected the PO₄ removal (Fig. S3).

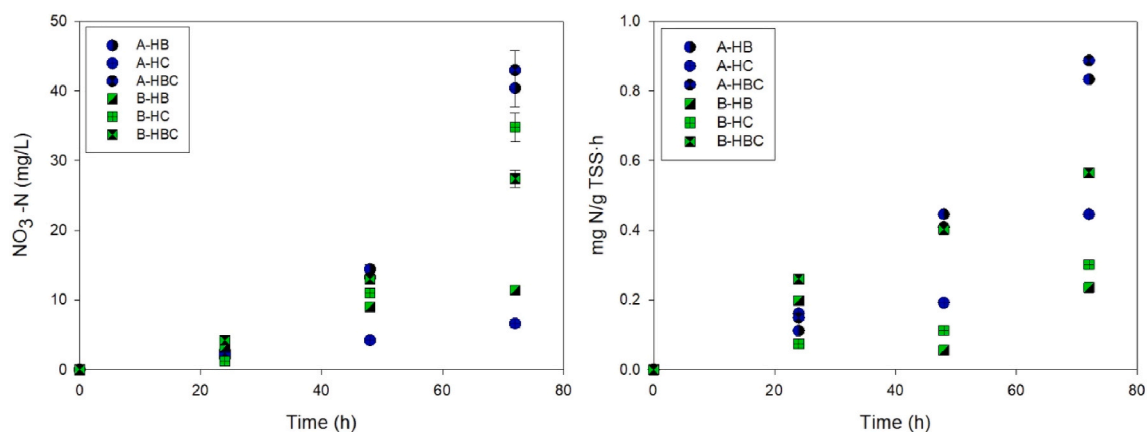


Fig. 2. Variation of NO₃-N concentration over time (Left) and nitrification rate (Right) in different hydrogels: HB: hydrogel with biomass, HC: hydrogel with AC, HBC: hydrogel with biomass and AC, exposed to medium A (synthetic wastewater) and medium B (synthetic wastewater with PFDA).

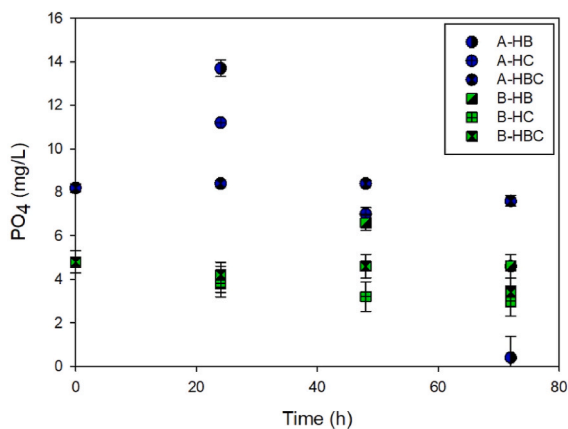


Fig. 3. PO₄ concentration over time in different hydrogels: HB: hydrogel with biomass, HC: hydrogel with AC, HBC: hydrogel with biomass and AC, exposed to medium A (synthetic wastewater) and medium B (synthetic wastewater with PFDA).

3.3. COD change

The concentration of COD increased for all hydrogels treating SWW (Fig. 4), which presented final concentration of 1163 mg/L, 988 mg/L and 1032 mg/L for HB, HC, and HBC, respectively. When hydrogels were exposed to SWW with PFDA, HB had a critical increase in COD concentration up to 1134 mg/L, followed by HC and HBC with 879 and 834 mg/L, respectively. This represents up to two times the initial COD concentration. In the case BOD concentration at the end of experiments, final concentration for HB and HBC treating SWW was below 68 mg/L, while for same hydrogels treating SWW in presence of PFDA, BOD concentration was an average of 125 mg/L. These results show that COD values were more related to the hydrogel composition, e.g., PVA, which presents very low biodegradability. For this reason, hydrogel beads were analyzed by TEM imaging to observe any damage or alteration in their internal structure.

3.4. PFDA removal

PFDA concentration was indirectly measured as F⁻ in SWW and DI, both containing 50 mg/L of PFDA (36 mg/L of F⁻). Fig. 5 shows that the highest removal was found for HC with 38.5%, followed by HB with 31%, while HB resulted in 30.2% of removal in the PFDA in DI water. Removal of PFDA in SWW was lower than in DI water, 27.7% with HC, 24.4% with HBC and 17.7% with HB. This indicates the inference that SWW constituents in the adsorption of PFDA, which was confirmed in the main effects plot (Fig. S6). Moreover, it was also noted that the presence of AC in hydrogels contributed to the removal of PFDA either in SWW and in DI water (Fig. S6), which can be attributed to its adsorption capacity.

3.5. Morphological change

To evaluate the morphological conditions of the synthesized hydrogels containing microalgae-bacteria biomass, TEM imaging was

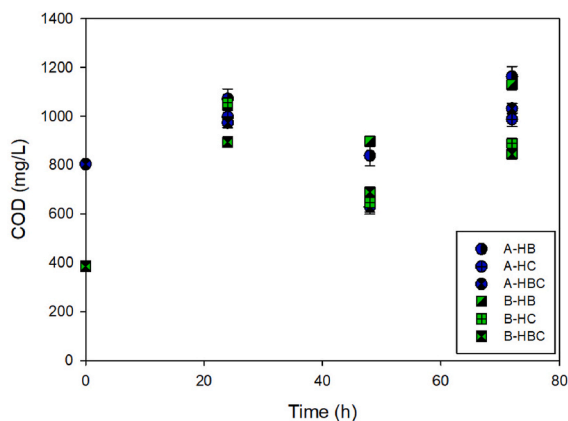


Fig. 4. Variation of COD concentration in different hydrogels: HB: hydrogel with biomass, HC: hydrogel with AC, HBC: hydrogel with biomass and AC, exposed to medium A (synthetic wastewater) and medium B (synthetic wastewater with PFDA).

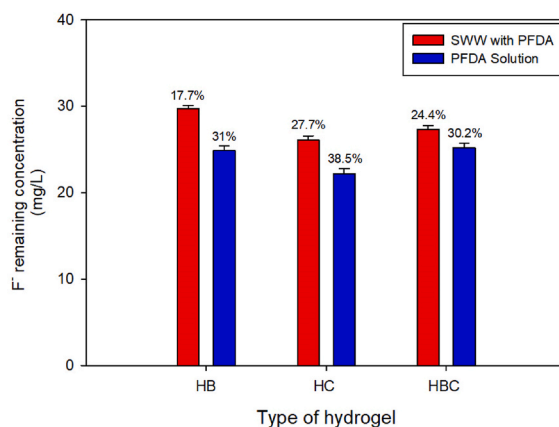


Fig. 5. Remaining concentration of F^- (mg/L) at end of experiments in SWW with PFDA (red) and in PFDA dissolved in DI water (blue). The removal percentage is presented on top of bars for each type of hydrogel. Hydrogel with biomass (HB), hydrogel with AC (HC), hydrogel with biomass and AC (HBC). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

performed in hydrogels without microorganisms (Fig. 6A) and in HB (Fig. 6B). Since processing of samples was performed with staining, the light color corresponds to the porosity of the hydrogel for Fig. 6A, as well as for the microorganism distribution within hydrogels in Fig. 6B. In the first case, the interconnection of internal microchannels is clearly observed (Green arrows), which are similar to those hydrogels prepared in the same basis of PVA-SA [45]. In Fig. 6B, the presence of microalgae is observed even with some vacuoles inside (Red arrows) and are very similar to *Scenedesmus* sp. and diatoms already reported [46]. Moreover, the presence of bacteria can be inferred for their size which is around 0.8–1.5 μm (Blue arrows). From the comparison of both figures, it is observed the successful embedding of microorganisms in hydrogel, and mechanical damage is not observed inside hydrogels, neither microorganisms' lysis. Therefore, the increasing COD removal is attributed to the PVA-SA leaching from hydrogels, which was confirmed when hydrogels were exposed to DI water (Fig. S5). This has been also observed in PVA-SA hydrogels when pH (3–5) and exposition time of up to 8 h increased [34]. In fact, it is expected that under low pH such as the PFDA solution (4.8), hydrogel lost microchannels used as cell proxies.

4. Discussion

Removal of $\text{NH}_4\text{-N}$ results (Fig. 1) indicated that regardless of the presence of PFDA, the hydrogels removed between 60 and 80%. However, the removal processes were different in the absence and presence of PFDA. In absence of PFDA, the effectiveness of hydrogels resulted from the $\text{NH}_4\text{-N}$ removal process by the microalgae-bacteria consortium. The symbiosis of these microorganisms has been studied and applied to accelerate the remediation of industrial [47] and domestic wastewater [48]. In this relation, the microalgae are

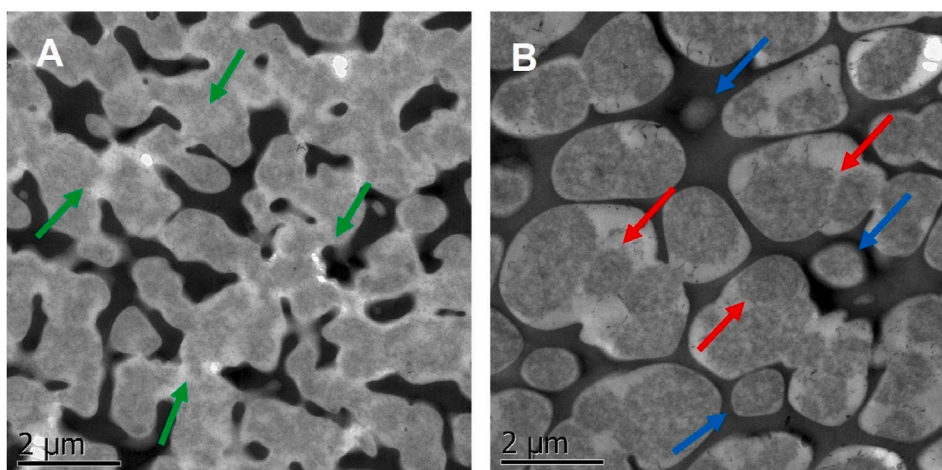


Fig. 6. Transmission electron microscopy (TEM) imaging of hydrogel structure without microorganisms (A) where green arrows indicate the microchannels; and hydrogels with microalgae-bacteria inside hydrogels (B), where red arrows stand for vacuoles in microalgae, and blue arrows for bacteria of the consortium. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

responsible for assimilating high loads of $\text{NH}_4\text{-N}$ and CO_2 to produce O_2 ; while the bacteria uptake the organic matter present in the medium and produces CO_2 and NO_3 . This symbiotic process of microalgae-bacteria for $\text{NH}_4\text{-N}$ assimilation has been reported to occur in SA beads [49], as well as in PVA + SA beads [50]. In the presence of PFDA, the removal of $\text{NH}_4\text{-N}$ can be attributed to AC due to low nitrate production. AC is commonly used as an adsorbent of contaminants in wastewater due to its characteristics [51], e.g., porous structure and high specific surface area. Therefore, $\text{NH}_4\text{-N}$ could have been adsorbed in AC, which has already been reported for commercial AC exposed up to 660 mg/L of $\text{NH}_4\text{-N}$ [52].

Nitrate production was the main process affected by PFDA. This process depends on the performance of microalgae and nitrifying bacteria for O_2 and NO_3 production, respectively. Both types of microorganisms have demonstrated sensitivity to PFAS, affecting their metabolic performance. In one hand, *Senedemus* sp is a green microalga used in our experiment that has presented an effective concentration 50 (EC50) of 44 mg/L for PFOA at 96 h of exposition, suppressing their metabolic activities for 8 days [53]. In the other hand, nitrifying bacteria have been affected by 1 mg/L at chronic exposure (48 days), decreasing 22.5% the nitrogen removal [29]; even the genes AOA and AOB amoA, related to nitrification process, have been affected by presence of PFAS [54]. However, based on the low DO concentration present in the hydrogels exposed to PFDA (Fig. S7), it can be inferred that the main adverse effect of PFDA occurred over microalgae metabolic activity. Therefore, it is reported for the first time that PFDA affects the nitrification process of microalgae-bacteria consortium immobilized and applied for wastewater treatment. This is a concern due to some CECs such as sulfamethoxazole, had non-effects during nitrogen removal using microalgae-bacteria consortium immobilized in SA beads [49].

Mass balance analysis on nitrogen consumption in SWW and SWW with PFDA indicated the negative effect of PFDA on nitrogen uptake by microalgae present in hydrogels. From the total nitrogen supplied, HB treating SWW uptake 23.17% of N, while in presence of PFDA there was a release of $\text{NH}_4\text{-N}$, presenting a negative value (-31.37%). The $\text{NH}_4\text{-N}$ increases in the balance in the aqueous media can be attributed to the releasing of some N sources, such as proteins, that can be measured as ammonium [55]. experimented with *Chlorella pyrenoidosa* and *Mycrocystis aeruginosa*, and exposed them to chlorotetracycline (CTC), which promoted proteins secretion to the aqueous media. This phenomenon is a common mechanism as a stress response.

Phosphate was removed at different levels by all hydrogels, although it was found that for SWW at 24 h, there was an increase in PO_4 concentration in all experiments. This effect may have resulted from the release of intracellular phosphate content from the hydrogel matrix to the medium [56], which was significantly reduced over time. The high PO_4 removal via HB (95%) can be attributed to the metabolism of microalgae, which have been applied in wastewater treatment to reduce the concentration of nutrients such as phosphorus [57–59]. This process can be improved with the implementation of bacteria [60]. reported that the nutrient absorption abilities of *Chlorella vulgaris* were improved with the presence of *Pseudomonas putida*, resulting in mutual interaction and synergetic effects. However, in presence of PFDA, HB resulted in the treatment with the lowest concentration of PO_4 removed, which is in line with the poor performance of microalgae caused by PFDA; meanwhile, HC and HBC achieved greatest removal. The presence of AC in the hydrogel was mainly considered as the principal reason for PO_4 removal. Various authors [61–63] have reported that AC as an excellent adsorbent of PO_4 in wastewater, which is mainly attributed to electrostatic interactions, physisorption and chemisorption.

The constant increase of COD concentration can be attributed to the leaching of PVA, which can be confirmed with the control experiment (hydrogels exposed to DI water). In that experiment, COD concentration began with 38 mg/L, while the final concentration resulted in 571, 508, and 856 mg/L for HB, HC, and HBC, respectively. In experiments exposed to PFDA, the missing fraction of COD can come from metabolic substrates such as soluble algal products (SAPs) [64]. These products are mostly organic matter released from microalgae to the medium; their source can be extracellular organic matter secreted from living cells, and intracellular organic matter released from dead and stressed cells [65].

The removal of PFDA in hydrogels can be considered as a positive outcome. Degradation of PFDA via microorganisms under this process conditions is quite difficult [66], therefore, its removal was attributed to adsorption process in hydrogels. The adsorption of PFAS, considered as an effective remediation technique [67], has been primarily attributed to hydrogen bonds between an adsorbent and the hydrophilic group of PFAS [68]. In our experiment, either polymeric matrix or AC can perform hydrogen bonds. Reversible hydrogen bonds have been observed in PVA hydrogels [69], while more stable bonds have been observed for AC [17]. Considering that highest removals of PFDA were observed for HC, it can be discriminated that adsorption onto AC was the main pattern in our experiment. Regarding media, PFDA removal was clearly better in the media with only PFDA, indicating that the presence of constituents of SWW such as anions, e.g., SO_4^{2-} , NO_3^- , PO_4^{3-} , and Cl^- , compete with PFDA in the adsorption process.

5. Conclusions

The present study demonstrated the negative impact of PFDA during the removal of nitrogen, phosphorus, and organic matter through hydrogels with a consortium of microalgae-bacteria within 72 h. The results obtained indicated that despite the impact of PFDA on the consortium, the nitrification process was carried out. Hydrogels with AC were the most efficient in removing more than 70% and 29% of $\text{NH}_4\text{-N}$ and PO_4 , respectively. In addition, PFDA was eliminated up to 27.7% mainly by adsorption in AC. It was concluded that the presence of AC in hydrogels can minimize the toxicity of PFDA over microalgae and bacteria consortium applied for wastewater treatment. Although this study evaluated concentrations higher than those detected in water bodies, the results could indicate that treatment systems exposed to chronic concentrations of PFDA would not remove this compound from wastewater either. Future perspectives include the study of the transport mechanism and flow of PFDA through the hydrogels, as well as the analysis of the growth kinetics of the consortium.

Author contribution statement

Marién Morán-Valencia, M.Sc.: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Carlos Huerta-Aguilar, PhD: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data ; Wrote the paper.

Abraham Mora, PhD; Jürgen Mahlknecht, PhD: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ayman N Saber, PhD: Analyzed and interpreted the data.

Pabel Cervantes-Avilés, Ph.D.: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pabel Cervantes Avilés reports financial support was provided by Tecnológico de Monterrey. Pabel Cervantes Avilés reports a relationship with Tecnológico de Monterrey that includes: employment. Pabel Cervantes Avilés and Marién Morán Valencia have a patent pending.

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Appendix A. Supplementary data

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